REMARKS

Claims 19-112 were previously pending. By this amendment claims 36, 49, 67, and 80 are canceled without prejudice or disclaimer. No new claims are added. No new matter has been introduced.

Applicant acknowledges that the examiner indicated on page 2 of the office action that Groups I and II have been rejoined. The examiner further indicated that the species requirement has been maintained and that as a result claims 24, 31, 34, 38, 45, 47, 55, *61*, 65, 69, 76, 78, 85, 88, *91*, 93, 95, 101, 104, 109 and 111 are withdrawn from further consideration as being drawn to nonelected species, there being no allowable generic or linking claim. Applicant respectfully traverses this listing of withdrawn claims in respect of claims 61 and 91.

It appears the examiner meant to withdraw claim 62 rather than claim 61. Claim 61 includes elected species GTCGTT and closely parallels claims 30, 84, and 100, none of which are withdrawn. Claim 62 relates to the sequence AACGTT and closely parallels claims 31, 85, and 101, all of which are withdrawn. Thus Applicant requests clarification and correction by the examiner in respect of the status of claims 61 and 62, because Applicant believes the examiner meant to indicate claim 62 is withdrawn rather than claim 61.

It appears the examiner did not mean to withdraw claim 91. Claim 91 recites a number of formulas for CpG motifs, including 5′-Purine-TCG-Pyrimidine-Pyrimidine-3′, which encompasses elected species GTCGTT. Claim 91 also closely parallels claims 83, 99, and 107, none of which is withdrawn. Thus Applicant requests clarification and correction by the examiner in respect of the status of claim 91, because Applicant believes the examiner did not mean to indicate claim 91 is withdrawn.

Assuming claim 62, rather than claim 61, is withdrawn and claim 91 is not withdrawn, claims 19-35, 37-48, 50-66, 68-79, and 81-112 are currently pending, of which claims 19-23, 25-30, 32, 33, 35, 37, 39-44, 46, 48, 50-54, 56-61, 63, 64, 66, 68, 70-75, 77, 79, 81-84, 86, 87, 89-92, 94, 96-100, 102, 103, 105-108, 110, and 112 are under examination.

Rejections Under 35 U.S.C. 112, First Paragraph (Enablement)

Claims 19-23, 25-30, 32, 33, 35-37, 39-44, 46,48-54, 56-61, 63, 64, 66-68, 70-75, 77, 79-84, 86, 87, 89, 90, 92, 94, 96-100, 102, 103, 105-108,110 and 112 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the enablement requirement.

According to the Examiner, the specification does not teach any of the methods as set forth in the instant claims for treating, preventing or ameliorating mycobacterium infections in a subject. According to the Examiner, "the specification teaches numerous in vitro experiments, however these data do not indicate enablement for the claimed invention."

The in vitro data presented in the specification is sufficient to support the claimed invention. Applicants have described a class of molecules (nucleic acids) having a common structural motif (a CpG dinucleotide) that when administered to a subject results in an aspect of the immune response being altered, with a Th1 response being favored. This class of oligonucleotides is described throughout the specification and their ability to produce an immune response is not only described (e.g., see page 8, lines 22-23 and 25-27, page 9, lines 8-9 and page 53, line 26 – page 54, line 5) but data is presented *in vitro* and *in vivo* using an adequate number of different CpG containing oligonucleotides to meet the enablement requirement for the claimed invention. The data in the application, including that represented in Tables 1-3, establishes that the unmethylated CpG is responsible for the immune stimulation. More than 40 oligonucleotides were tested. The data represented in Table 5 demonstrates that the immune stimulation has the characteristic pattern of a Th1 response. Eleven different oligonucleotides induced a Th1 cytokine profile, demonstrating the consistent stimulatory effect of CpG containing oligonucleotides.

On page 35 of the specification under the heading "Teleological Basis of Immunostimulatory Nucleic Acids" it is taught that the stimulatory CpG motif, identified according to the invention, is common in microbial genomic DNA, but quite rare in vertebrate DNA. Experiments described in Example 3, in which methylation of bacterial DNA with CpG

methylase was found to abolish mitogenicity, demonstrated that the difference in CpG status is the cause of immune stimulation by bacterial DNA. It is further taught that "Teleologically, it appears likely that lymphocyte activation by the CpG motif represents an immune defense mechanism that can thereby distinguish bacterial from host DNA."

The specification includes *in vitro* data on mouse and human cells, as well as *in vivo* data. Tables 1-3 demonstrate that many different CpG oligonucleotides are capable of activating murine B cells and inducing cytokine expression in murine cells *in vitro*. Table 5 depicts an experiment in which multiple CpG containing oligonucleotides were tested for their ability to induce cytokine expression in human cells. The experiment of Table 5 demonstrated that multiple CpG oligonucleotides were capable of inducing cytokine expression.

The Examiner has cited several papers in support of the lack of enablement rejection and in particular in support of the argument that the state of the art at the time of the invention was unpredictable.

In particular, the Examiner has stated that "the history of vaccination in humans (the scope of the instant claims) against Mycobacterium disease (tuberculosis) is notorious for lack of a successful protection (i.e. prevention) as well as amelioration." The examiner cites Wiegeshaus, E.H. et al, Reviews of Infectious Diseases, April 1989, 11/Suppl. 2:S484-S490) for the teaching that animal models used to evaluate the relative protective potency of a panel of tuberculosis vaccines have yielded dissimilar data. "Wiegeshaus et al teaches that animal models have produced disparate data on the protective potency of tuberculosis vaccines, therefore the variables comprising such models cannot be randomly chosen and that it is not known which animal model, if any, predicts the protective potency of vaccines for humans (page S490)." (Office Action page 4).

Wiegeshaus is a review article summarizing the state of research on vaccine adjuvants for tuberculosis in 1989. The reference does not describe CpG nucleic acids as adjuvants. The focus of the article is on how results of animal models can be used to correlate with human treatments in this disease state. There are always limitations on the predictability of animal models for human disease. This does not render the use of CpG oligonucleotides as vaccine adjuvants unpredictable.

The Examiner has cited Griffin et al (Trends in Microbiology, Nov. 1995, 3/11: 418-424) for the teaching that "limitations in the design of field studies have seriously compromised our ability to evaluate the efficacy of bacilli Calmette-Guerin (BCG) in human and animal populations accurately. Griffin et al teaches that humans, cattle, deer, guinea pigs and rabbits have similar pathology but differ in their susceptibility to tuberculosis (p. 418)." It is further stated that "Griffin et al further teach that many critical factors that are required to generate protective immunity and target the cellular pathways for appropriate T cell activation and effector activity against tuberculosis have been identified and the ability to exploit this knowledge depends on relevant animal models being available to test new candidate vaccines (pages 422-423)." (Office Action pages 4-5).

Griffin et al provide a summary of research studies conducted on vaccines in several animals. They conclude that deer are a useful model for vaccination strategies for M. Bovis. Like Wiegeshaus, Griffin et al does not describe the use of CpG oligonucleotides as adjuvants in this system. A general teaching regarding animal models for vaccines generally is not sufficient to establish the unpredictability of CpG nucleic acids as vaccine adjuvants.

McCluskie et al 1999 and Krieg et al 2000 have been cited for the proposition that biological responses to the administration of CpG containing oligonucleotides vary depending on the mode of administration and the organism.[1]

McCluskie et al is an article describing DNA vaccines against Hepatitis B virus. On page 296, the page identified by the examiner, the reference mentions that one of the factors involved in influencing the Th bias of the response to DNA vaccines is the presence of CpG motifs. The reference, however, is not relevant to the enablement of the pending claims because the pending claims do not encompass DNA vaccines. The pending claims are directed to the use of nucleic acids that are immunostimulatory. The methods of the invention do not involve expression of antigens from plasmid vectors. The issues of predictability and therapeutic effectivity are very different for immunostimulation using CpG nucleic acids and DNA vaccines.

[1] The Examiner has not provided a citation for Krieg and McCluskie. Applicants assume that the Examiner is referring to the following citations, based on similar rejections in related applications: McCluskie et al 1999 (Molecular Med. 1999, 5/5:287-300) and Krieg et al 2000 (Immunology Today 2000, 21/10:521-526). However if the Examiner intended to refer to different citations she is asked to clarify the record.

Krieg and Wagner is a review article describing the uses of CpG oligonucleotides. The office action specifically points to page 524 of the reference in support of the examiner's argument that biological responses to the administration of CpG containing oligonucleotides vary depending on the mode of administration and the organism. Applicants do not see this teaching in the reference. In fact the reference teaches on page 524 that "Unlike many vaccine adjuvants that have been extremely effective in mice but disappointing in humans, CpG DNA is also highly effective in higher primates." This teaching does not support the examiner's assertion that the administration of CpG oligonucleotides varies depending on the organism. Furthermore, Krieg et al describe the usefulness of CpG oligonucleotides in producing a Th1 biased immune response. Page 524 of Krieg et al includes the following teaching:

> "These and subsequent studies have shown CpG DNA to be a more effective Th1-like adjuvant than complete Freund's, and to be effective with multiple types of antigens and routes of immunization including mucosal immunization (reviewed in Ref. 50). In fact, in a comparison of 19 different adjuvants, CpG DNA was found to be the strongest for inducing Th1-like immune response to tumor antigens[51]" and
> "The potent Th1 adjuvant effect of CpG can even override preexisting Th2 immune responses [5, 47]; it has been used as an adjuvant for allergy vaccines, where it induces Th1 responses to antigens in the presence of a preexisting Th2 response, leading to decreased symptoms following subsequent allergen inhalation ..... It should be stressed that CpG DNA is effective in asthma immunotherapy even when given as a stand-alone agent without allergen." [Emphasis added]

Weiner has also been cited for the proposition that the molecular mechanisms of CpG oligonucleotides' immunostimulatory effects are not yet understood (referring to page 461).[2] It is also stated that Weiner suggest that the clinical effects of CpG ODN have not yet been explored and further work with the immunostimulatory nucleic acids in-both the laboratory and the clinic are needed before their true promise as investigational immunological and therapeutic agents is known.

[2] It is believed that the rejection is referring to Weiner, J. Leukocyte Biology, 2000, 68:456-463.

Knowledge of the mechanism of action isn't necessary, particularly in view of the detailed knowledge at the time the patent application was filed of the cellular effects of CpG oligonucleotides. The patent application identifies consistent changes in the immune system at the cellular level that occur in response to CpG administration and which are therapeutically relevant. Additionally, Table 1 of Weiner lists examples of cellular effects arising from immunostimulatory CpG ODN. A lack of understanding of the molecular mechanism does not render the cellular results unpredictable. Other statements in Weiner are consistent with enablement of the claimed invention. For instance it is taught on page 456 1st column second full paragraph that "Studies to date suggest CpG DNA could have significant therapeutic promise in the treatment of a variety of disorders, including infectious disease, allergy, and cancer." Page 457 under "In vivo effects of CpG ODN" teaches that "extensive studies have been done in rodents, and some studies have been done in non-human primates. The observed *in vivo* data fits well with the *in vitro* data outlined above."

Agrawal et al (Molecular Med. Today 2000, 6:72-81) has been cited in support of the assertion that "chemical modifications have been studied for CpG containing oligonucleotides, such as 2'-0-methyl modifications, phosphorothioate internucleotide linkages and 5-methyl cytosine substitutions, the incorporation and positioning of chemical modifications relative to the CpG dinucleotide are highly unpredictable." In particular, the examiner has identified pages 78-80 as being particularly relevant. Agrawal et al is a review article describing antisense oligonucleotides. The authors suggest on page 78 that in order to *reduce* non-antisense related activity it is best to avoid CpG motifs. The authors also indicate that if it is not possible to avoid CpG motifs, then it is possible to make one of 3 modifications to reduce the CpG activity of the oligonucleotide. One of the suggested modifications is to replace the cytosine base of the CpG with a 5-methyl cytosine base. The instant specification teaches that a CpG containing oligonucleotide has an unmethylated C in the CpG motif. Further, the cited section of Agrawal et al teaches that the proposed 3 modifications "significantly reduced side effects". Agrawal et al does not teach that immune stimulation was abolished with any of these proposed modifications, just reduced.

O'Hagan et al 2001 is cited for the teaching that CpG ODN cannot be used without being conjugated to an antigen. According to the Examiner, O'Hagan "does teach that the CpG has adjuvant properties and that this effect appears to be maximized by their conjugation to protein antigens or their formulation with delivery systems (p. 75)." (Office Action page 7).

O'Hagan et al is a review article describing various research studies on adjuvants for vaccines. Immunostimulatory adjuvants, including CpG ODN are described on pages 74-76. O'Hagen et al teach that CpG ODNs "appear to have significant potential as mucosally administered adjuvants" (Page 74, 1st column, 2nd paragraph) and that "potent adjuvant effect have been achieved in human clinical trials (Heather Davis, unpublished observations)" (Page 74, 1st column, 2nd paragraph). The instant specification teaches that CpG nucleic acids can be administered in conjunction with antigens. However the CpG may also be administered without an antigen. O'Hagan doesn't teach that CpG administered without being conjugated to an antigen doesn't work. In fact the cited clinical trials in humans do not involve conjugated antigen. O'Hagen et al, simply teach that in some systems CpG nucleic acids conjugated to antigen have good results. Thus, O'Hagen et al do not teach that immune stimulation was abolished without conjugation, just that conjugation may help to maximize results.

The Examiner concludes that "Applying the above test to the facts of record, it is determined that 1) no declaration under 37 C.F.R. 1.132 or other relevant evidence has been made of record establishing the amount of experimentation necessary, 2) insufficient direction or guidance is presented in the specification using the claimed methods to treat Mycobacterium infections (tuberculosis) in subject administering a CpG immunostimulatory nucleic acid molecule as previously stated, 3) there are no working examples presented in the specification that teach the claimed methods to treat, prevent or ameliorate Mycobacterium infections as previously stated, 4) the relative skill of those in the art is commonly recognized as quite high (post-doctoral level), and 5) the state of the art in the field to which the invention pertains is recognized in the art as evidenced by the cited prior art.

As described above, numerous working examples and data were provided in the specification. These examples in combination with the description in the specification were sufficient to enable one of skill in the art to practice the invention over the full scope of the

claims. Consistent with these descriptions, a number of studies published since the filing of the patent application have reiterated, as set forth in the specification, that CpG oligonucleotides having different structures but maintaining the critical CpG motif result in an altered immune response. Applicant has addressed the supposed unpredictability associated with the prior art discussed above.

Thus, one of ordinary skill in the art, based on the teachings in the patent application, would have reasonably expected the claimed invention to work over the full scope of the claims.

Rejections Under 35 U.S.C. 112, Second Paragraph

Claims 22, 36, 49, 53, 67 and 80 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, claims 22 and 53 are rejected as being vague and indefinite in the recitation of "sufficient immunostimulatory motifs to be immunostimulatory". The limitation is not indefinite. Applicants do not need to provide a specific number of CpG motifs within the molecule that make it immunostimulatory. Rather, the claim limitation requires that the molecule have enough to make it immunostimulatory. One of skill in the art can identify the appropriate immunostimulatory sequences based upon Applicants descriptions in the specification.

Claims 36, 49, 67 and 80 have been rejected as being vague and indefinite in the recitation of "immune system is not functioning in a normal capacity." One of ordinary skill in the art would understand the meaning of the term. When used in the context of an immune system the term "normal" refers to a healthy subject. This is the ordinary meaning of the term as it is known in the art. The term does not render the claim indefinite. However, in the interest of expediting prosecution, these claims have been canceled.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

By _____

Helen Lockhart
Registration No.: 39,248
WOLF, GREENFIELD & SACKS, P.C.
Federal Reserve Plaza
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
(617) 646-8000